Terpenes for Indirect Selection of Growth Potential in Rocky Mountain Douglas-Fir

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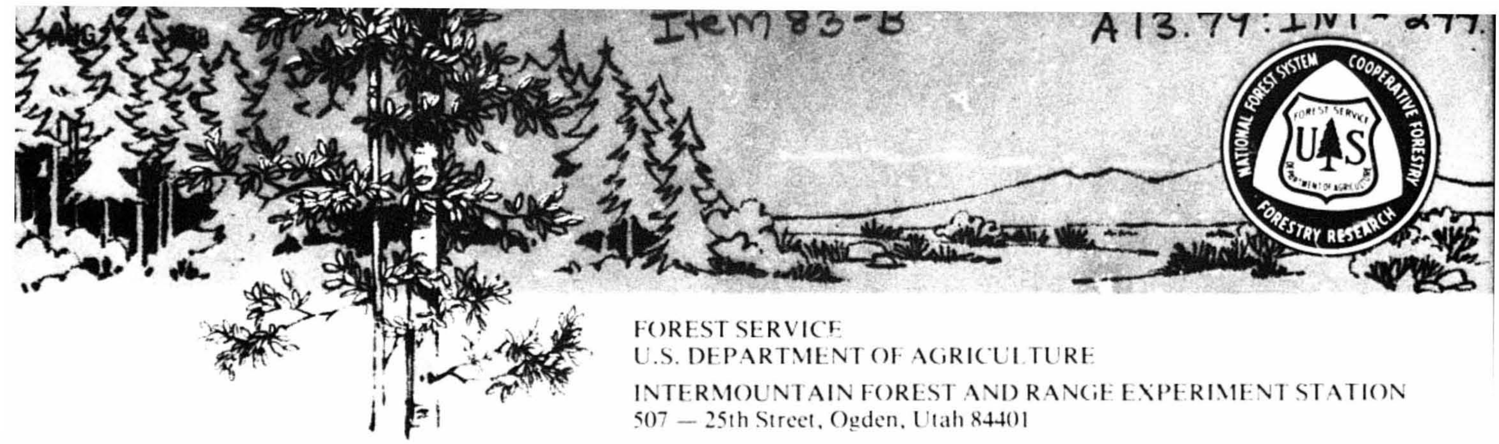

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TERPENES FOR INDIRECT SELECTION OF GROWTH POTENTIAL IN ROCKY MOUNTAIN DOUGLAS-FIR

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ABSTRACT

Possibilities of using terpene composition for indirect selection of growth potential were explored for Rocky Mountain Douglas-fir. Volatile leaf oil analyses were made on 170 5-year-old seedlings that represented full-sib hybrid families, wind-pollinated parental lines of inland origin, and wind-pollinated parental lines of coastal origin. Correlation analyses showed that terpenes could not be used efficiently for indirect selection of growth potential in the inland variety. But, terpenes may be useful in selecting parental lines in programs of intervarietal hybridization.

KEYWORDS: *Pseudotsuga menziesii*, terpenes, indirect selection, growth potential

Because tree growth is a product of genotype and environment, the genotype of a tree is often poorly expressed phenotypically. In many instances it would be desirable to identify trees of superior growth potential from morphological, physiological, or biochemical traits that are not under strong environmental influence. Such indirect selection would facilitate rapid incorporation of genetics research into management options. For example, seed orchards could be stocked without progeny tests, and trees from which natural regeneration is to be encouraged could be identified readily.

Unique possibilities for indirect selection of growth potential exist in the inland variety of Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) in northern Idaho. First, the inland variety and the coastal variety (*P. menziesii* var. *menziesii*) are readily classified according to the terpenes contained within volatile leaf oils (von Rudloff 1972). Terpene concentrations have proven so useful diagnostically that von Rudloff (1973a, 1973b) recognized three coastal types; a single inland type; and three types that bridge the varieties: intermediate, coastal intermediate, and interior intermediate. Moreover, trees that express varying degrees of intermediacy in terpene

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omposition are occasionally found in inland populations (von Rudloff 1973b). Since nvironmental conditions have little effect on terpenes (von Rudloff 1972), these nland trees of intermediate terpene composition presumably contain genes for terpene roduction that are of coastal origin.

Secondly, it is well known that the growth potential of the coastal variety is uperior to that of the inland variety. Yet, northern Idaho populations are from an rea that represents a floristic transition from the maritime coastal forests to Rocky lountain forests (Daubenmire and Daubenmire 1968). The growth potential of populations from northern Idaho is generally superior to that of western Montana populations (Rehfeldt 1974). This difference in growth potential may reflect a higher frequency of genes typical of the coastal variety in populations from northern Idaho than in those from Montana.

Thus, possibilities of using terpenes for indirect selection of growth potential arise from concomitant variation in both traits. Can individual trees of the inland variety in northern Idaho be selected for high growth potential according to the relative concentration of those terpenes that typify the coastal variety? Although Hanover and Furniss (1966) found no relationship between the growth of 94 Douglas-fir trees and concentrations of four terpenes, the populations they sampled were from central Idaho and west-central Montana--geographical areas that are far removed from current zones of introgression between the two varieties.

If terpenes are usable for indirect selection of growth potential, terpene composition and rate of growth must be interdependent. This will happen only if terpenes and growth rate are (1) pleiotropic or (2) not at linkage equilibrium. Pleiotropic effects, the expression of dissimilar traits from the same genetic mechanism, require that the traits be correlated nearly perfectly. Linkage occurs between genes located on the same chromosome. At linkage equilibrium, the occurrence of genes at linked loci is independent. Equilibrium between linked genes is approached slowly (the average number of generations to reach equilibrium = reciprocal of the recombination frequency). Thus, even though introgression between the two varieties may have progressed for many generations, closely linked genes may not have reached equilibrium.

METHODS

Leaf oil analyses were made on 170 5-year-old seedlings growing in northern Idaho. These seedlings had been included in tests of intervarietal hybridization and represented 10 families: 4 full-sib hybrid families; 4 wind-pollinated parental lines of inland origin; and 2 wind-pollinated parental lines of coastal origin (tabl[illegible]). The cultural conditions under which the seedlings were raised and the nursery performance of hybrids and parental lines are documented elsewhere (Rehfeldt 1977). The height of all seedlings was measured at age 4. One year later, leaves were collected for analyses of leaf oils.

After dipping into liquid nitrogen, frozen leaves were stripped from the branches and stems. Leaves were steam-distilled for 5 h and the volatile oil was recovered as described by von Rudloff (1972). Aliquots (0.5 to 1.0 μl) of the volatile oil obtained from each seedling were analyzed on four different gas-liquid chromatographic (g.l.c.) columns (von Rudloff 1976a). The relative percentages of each terpene were determined by area integration of the g.l.c. peaks (sum of all peaks for each sample = 100 percent) with a Hewlett-Packard model 3352 data system. Results from the four columns were averaged. The g.l.c. error was 0.1 to 0.2 percent for well-resolved peaks and 0.3 to 1.0 percent for overlapping ones (see also von Rudloff 1973a). Peak identities had been established previously.

Table 1.--*Origin of families and average terpene classification of progenies. Classifications are based on techniques developed by von Rudloff (1972)*

Family code	♀ parent	♂ parent	General terpene classification of progenies	Number of seedlings
0	[1] 7	wind	interior intermediate	16
1	[1] 16	wind	interior	17
2	[1] 19	wind	interior	17
3	[1] 25	wind	interior	16
4	7	98	coastal intermediate	20
5	19	98	interior intermediate	16
6	16	100	interior intermediate	19
7	25	100	intermediate	20
8	[2] 98	wind	coastal	16
9	[2] 100	wind	coastal	13

[1] Identification number of parent tree growing near Clarkia, Idaho.

[2] Identification number of parent tree growing near Lake Cowichan, B.C. The authors gratefully acknowledge the assistance of A. Orr-Ewing and J. C. Heman, British Columbia Forest Service, Victoria, B.C.

The terpene compositions of individual seedlings and families were compared according to groups of biogenetically related terpenes (von Rudloff 1976b) that are defined in table 2. These comparisons were based on principles established previously (von Rudloff 1972, 1976b): seedlings having terpene compositions typical of the coastal variety have high levels of β-pinene, the sabinenes, and the terpinenes; seedlings having high levels of limonene and the camphene group are typical of the inland variety. Finally, the term "flavor" is used to describe terpene compositions that vary in the direction of those patterns that typify either variety.

Simple linear correlation coefficients were calculated between seedling height and the relative percentage of each terpene. Because of heterogeneous variances and skewed frequency distributions, height measurements were transformed to logarithms and percentages were transformed to $\sqrt{x + 1/2}$ before calculating correlation coefficients (Steel and Torrie 1960). Associations between terpenes and growth rate were interpreted according to effects of either pleiotropism or linkage.

Table 2.--*Family means for height (cm) and concentrations (percent) of terpenes summed according to groups of biogenetically related terpenes (von Rudloff 1976b)*

Family	α-pinene	β-pinene	Sabinene group[1]	Terpinene group[2]	Camphene group[3]	Citronellol group[4]	Limonene	Height
	Precursor group							
	Percent							*Cm*
Inland								
7 x wind	12.0	7.2	1.5	2.0	57.1	7.2	4.5	41.7
16 x wind	11.5	3.3	0.1	1.3	63.8	5.6	4.8	46.3
19 x wind	11.6	3.8	0.2	1.3	66.1	4.8	5.1	40.0
25 x wind	11.7	4.6	0.5	1.6	59.8	9.6	4.2	32.1
Hybrid								
7 x 98	14.2	39.0	5.7	4.2	17.7	7.7	1.9	82.6
19 x 98	12.5	12.5	0.8	1.5	55.1	6.3	4.1	45.2
16 x 100	12.8	10.1	0.6	1.3	53.4	8.2	4.3	59.7
25 x 100	12.1	16.0	1.3	1.9	41.4	14.6	3.5	66.6
Coastal								
98 x wind	14.3	55.2	1.3	1.7	1.2	14.3	1.2	62.1
100 x wind	13.5	49.3	1.2	1.7	1.0	18.5	1.6	66.8

[1]α-thujene + sabinene
[2]α- and γ-terpinene + terpinolene + terpinen-4-ol
[3]santene + tricyclene + camphene + borneol + bornyl acetate
[4]linalool + citronellol + citronellyl acetate + geranyl acetate

RESULTS AND DISCUSSION

The results of terpene analyses were unexpected. These results are expressed as an average terpene composition for each family (table 1) according to techniques of classification developed by von Rudloff (1972). In general, all wind-pollinated progenies of coastal origin (families 8 and 9, table 1) exhibited terpene compositions that are typical for coastal British Columbia. However, the wind-pollinated progenies of only two inland trees (families 1 and 2) expressed terpene compositions that were typical of the interior variety. A few wind-pollinated progenies of tree 25 (family 3) had terpene compositions of slight coastal flavor, but those of tree 7 (family 0) were extremely variable.

In fact, classification of terpenes of individual seedlings within wind-pollinated offspring of tree 7 revealed typical interior patterns, interior intermediate patterns, and even intermediate patterns. Mean values (table 2) show that an average wind-pollinated progeny of tree 7 had higher levels of β-pinene, the sabinenes, and the terpinenes, and lower levels of limonene and the camphenes than progenies of the other three interior trees. Consequently, it appears that tree 7 and perhaps tree 25 are segregating for genes controlling terpenes typical of the coastal variety.

The degree of coastal flavor to the classification of terpenes in families of hybrid origin follows patterns expected from the classification of parental lines (tables 1 and 2). However, classifications of all hybrid progenies show a greater interior flavor than that expected from an average of parental classifications.

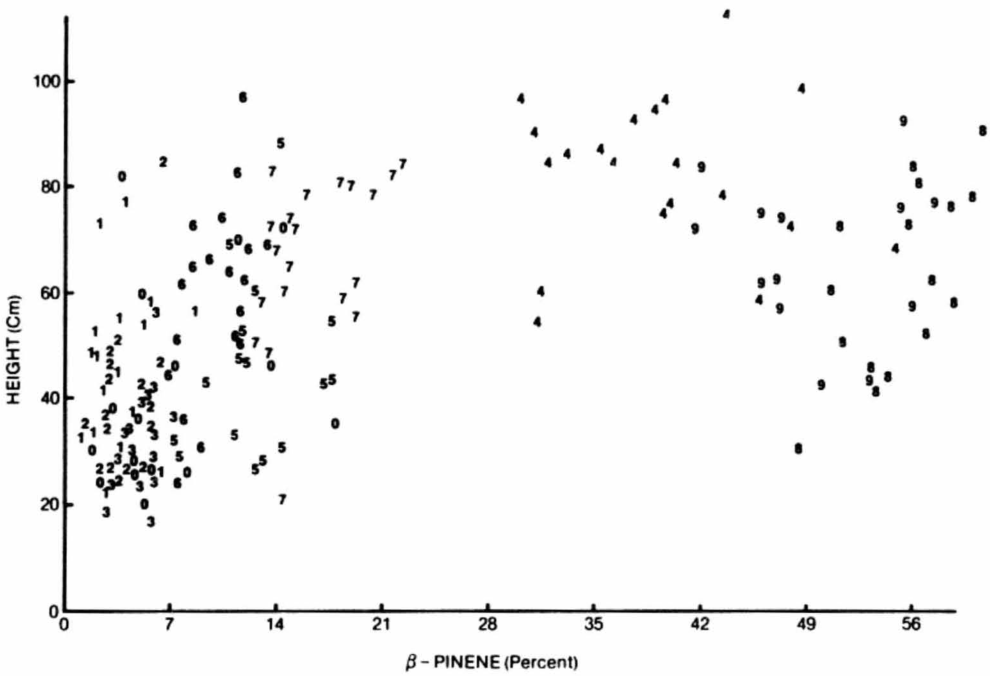

Figure 1.--Relationship between the height of individual seedlings and the percentage of β-pinene found among the total terpene concentration. Plotted symbols reflect family codes keyed to table 1.

Although mean heights of families of interior origin differed by about 14 cm (table 2), individual seedlings differed by as much as 60 cm (fig. 1). Unlike the terpene classifications, the mean heights for hybrid families are not predictable from the mean heights of parental lines. Yet, the results are encouraging because large amounts of variation in seedling height and in terpene composition for seedlings of interior origin increase the possibilities of detecting relationships suitable for indirect selection.

Correlation coefficients were calculated for the entire data set and for three subsets involving seedlings of interior, hybrid, and coastal origin. Correlations based on the entire data set show numerous statistically significant relationships between individual terpenes and height (table 3). Yet, as illustrated in figure 1 for β-pinene, most of these relationships reflect intrinsic differences between the varieties. In general, seedlings of hybrid or coastal origin were taller than those from the interior. All terpenes that are characteristically high in the coastal variety had a positive relationship with seedling height; those terpenes that are typically high in the inland variety had a negative relationship with height. Thus, correlations involving data from all seedlings contribute little toward understanding genetic relationships between terpene composition and growth.

Correlations involving seedlings of coastal or interior origin should express the genetic relationships between terpene composition and growth potential more clearly. But the results suggest a lack of genetic correlation (table 3). First, no significant relationships were found for seedlings of the coastal variety. Admittedly, the number of observations was small, and the seedlings were poorly adapted for the inland environment in which they were growing. Secondly, most of the significant relationships indicated for the interior variety (table 3) involve terpenes (α-pinene, citronellol, citronellyl acetate, and geranyl acetate) that do not differ to any large extent between

Table 3.--*Simple correlation coefficients between seedling height and terpene concentration*

Leaf oil	All data (170 seedlings)	Inland variety (66 seedlings)	Hybrids (75 seedlings)	Coastal variety (29 seedlings)
	Data set			
Santene	[1] -0.52*	-0.08	-0.53*	-0.05
Tricyclene	- .42*	.33*	- .47*	- .10
α-pinene	.55*	.35*	.40*	.37
Camphene	- .43*	.30	- .45*	.01
β-pinene	.56*	.16	.53*	.11
Sabinene	.46*	.21	.44*	.04
Myrcene	.44*	.03	.47*	- .27
3-carene	.16	.30	.17	- .25
α-terpinene	.34*	.19	.38*	.05
Limonene	- .48*	.00	- .45*	- .08
β-phellandrene	.53*	.13	.49*	- .07
Ocimene	- .27*	.11	.10	.29
γ-terpinene	.27*	.13	.33*	.27
Terpinolene	.27*	.19	.36*	- .11
Linalool	.10	.01	.02	.17
Unidentified A	.15	.01	.08	.13
Unidentified B	.11	.30	.12	.28
Borneol	.10	.26	.09	.12
Terpinen-4-ol	.34*	.00	.36*	.06
α-terpinol	.28*	.24	.22	.18
Citronellol	.29*	.34*	.04	- .19
Bornyl acetate	- .47*	- .06	.51*	- .04
Citronellyl acetate	.07	.57*	.15	- .06
Geranyl acetate	.12	- .40*	.32*	- .16

[1]*Statistical significance at the 1% level of probability.

varieties. Finally, a positive relationship in inland seedlings was indicated for tricyclene in a situation where a negative relationship would have indicated a genetic correlation. A negative relationship is expected because tricyclene, a member of the camphene group, is at relatively high concentrations in the inland variety. Consequently, if rapid growth is associated with terpene composition of coastal flavor, a negative relationship would be expressed.

Low values of the correlation coefficients within the two varieties imply that pleiotropism does not govern relationships between terpene composition and growth rate. Since pleiotropism involves the expression of dissimilar traits from the same set of genes, pleiotropic effects would be discernible in nearly perfect relationships.

Correlation coefficients for coastal and inland seedlings suggest that genes controlling terpene composition and growth potential are segregating independently. This conclusion is also supported by the wind-pollinated progenies of tree 7, the interior tree segregating for typically coastal terpenes. Both tall and short seedlings produced relatively high or low percentages of β-pinene (fig. 1, family 0). A similar relationship between height and the concentration of the other typically coastal terpenes was also evident within the wind-pollinated progenies of tree 7. Thus, coastal linkage groups involving rapid growth and high concentrations of β-pinene, the sabinenes, and the terpinenes are not evident in the interior variety.

On the other hand, correlations involving the hybrid data set (table 3) do not support independent segregation. Individual seedlings of hybrid origin had terpene compositions that ranged from interior types to coastal types. And, a positive relationship existed between seedling height and the degree of coastal flavor to the terpenes (table 3). Yet, figure 1 shows that these correlations result from family means rather than from correlations among individuals within families: the tallest hybrid family had terpenes of a distinctly coastal flavor; the shortest families had terpenes of interior flavor.

The relationship between mean height for hybrid families and terpene composition does not appear to be accidental. Tree 7, the interior tree segregating for coastal terpenes, combined with tree 98 to produce hybrids with terpenes that ranged from coastal intermediate to coastal types. Of the 90 hybrid families included in the original tests (Rehfeldt 1977), the seedlings produced from this cross were the tallest. Moreover, tree 25, identified as segregating for some terpenes of coastal flavor, produced hybrids that were slightly above average in height and intermediate in terpene composition. Trees 16 and 19, segregating for no coastal terpenes, produced hybrids that expressed a resemblance to the interior variety for both growth and terpene composition. Thus, the mean height for a hybrid family was directly related to the percentage of the typically coastal terpenes within the interior parental line. But, the mean height of the actual parental lines was not related to the segregation of genes for typically coastal terpenes (table 1). Are these results feasible biologically?

The data suggest that linkage equilibrium in the interior variety has been approached, but not achieved. In addition to genes controlling terpenes, tree 7 undoubtedly has small linkage groups that contain genes typical of the coastal variety. From analyses of the nursery performance of intervarietal hybrids, Rehfeldt (1977) concluded: "Interracial hybridization combines two differentially coadapted genetic systems. The performance of individual hybrid families depends not only on the degree to which specific parental genotypes integrate but also on the degree to which specific gametes integrate." Thus, the mean growth of a hybrid family is related to the proportion of the germ plasm that is of coastal origin. Because tree 7 had more linkage groups of coastal origin than any of the other inland trees, gametes from tree 7 integrated best with gametes of the coastal variety. The result was balanced, coadapted hybrid genotypes (family 7X98) with a strong resemblance to the coastal variety. on the average, these hybrid families should express relationships between growth rate and degree of coastal flavor to their terpenes. In addition, if linkage groups of coastal origin are approaching equilibrium, a relationship between growth rate and terpene composition would not be expected in wind-pollinated progenies of inland trees, particularly if genes of coastal origin are at low frequencies. Consequently, because of a lack of correlation within families, it seems advisable to assume that linkage equilibrium has been achieved between growth rate and terpene composition of the inland variety.

CONCLUSIONS

Practical implications of these results are limited. Because linkage seems to be near equilibrium, the use of terpenes for indirect selection of fast-growing inland genotypes would be inefficient and impractical. However, tests of intervarietal hybrids (Rehfeldt 1977) suggest a high potential of hybridization for improving the growth rate of the inland variety while maintaining cold hardiness. Chemical analyses of leaf oils may provide a rapid means of selecting genotypes of the inland variety that will combine best with coastal gametes.

PUBLICATIONS CITED

Daubenmire, R., and J. B. Daubenmire.
1968. Forest vegetation of eastern Washington and northern Idaho. Wash. Agric. Exp. Stn. Tech. Bull. 60, 104 p.

Hanover, J. M., and M. M. Furniss.
1966. Monoterpene concentration in Douglas-fir in relation to geographic location and resistance to attack by the Douglas-fir beetle. *In*: Soc. Am. For. 2nd Genet. Workshop and Lake States For. Tree Improve. Joint Proc., p. 23-28. USDA For. Serv. Res. Pap. NC-6, 110 p. North Cent. For. Exp. Stn., St. Paul, Minn.

Rehfeldt, G. E.
1974. Genetic variation of Douglas-fir in the Northern Rocky Mountains. USDA For. Serv. Res. Note INT-184, 6 p. Intermt. For. and Range Exp. Stn., Ogden, Utah.

Rehfeldt, G. E.
1977. Growth and cold hardiness of intervarietal hybrids of Douglas-fir. Theoret. Appl. Genet. 50:3-17.

Steel, R. G. D., and J. H. Torrie.
1960. Principles and procedures of statistics. 481 p. McGraw-Hill, New York.

von Rudloff, E.
1972. Chemosystematic studies in the genus *Pseudotsuga*. I. Leaf oil analysis of the coastal and Rocky Mountain varieties of the Douglas-fir. Can. J. Bot. 50:1025-1040.

von Rudloff, E.
1973a. Chemosystematic studies in the genus *Pseudotsuga*. II. Geographical variation in the terpene composition of the leaf oil of Douglas-fir. Pure Appl. Chem. 34:401-410.

von Rudloff, E.
1973b. Chemosystematic studies in the genus *Pseudotsuga*. III: Population differences in British Columbia as determined by volatile leaf oil analysis. Can. J. For. Res. 3:443-452.

von Rudloff, E.
1976a. Chemosystematic studies in the genus *Abies*. II. Leaf oil analysis of grand fir. Can. J. Bot. 54:1926-1931.

von Rudloff, E.
1976b. Inheritance of leaf oil terpene patterns in coastal and interior Douglas-fir populations and some of their crosses. IUFRO Adv. Tree Breed. Symp., Bordeaux, 1976.